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Cell-penetrating peptides: Enhancing antimicrobial efficacy against intracellular pathogens

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Abstract

Cell-penetrating peptides (CPPs) are naturally occurring or synthetic peptide sequences that possess the inherent capability to penetrate various cell types and concurrently carry hydrophilic substances and nanomaterials, for which cellular absorption is typically restricted. Alongside this fundamental action of cell entry without damaging the membrane, certain CPPs exhibit multivalent antimicrobial properties. The method they utilize to penetrate cells continues to be an unresolved aspect of the mystery. Direct translocation and endocytosis have been proposed as the primary mechanisms for internalization; however, vesicle budding and collapse have also been mentioned. CPPs exhibiting antimicrobial properties (CPAPs) represent a distinct group of bioactive peptides that emerge as potential solutions for combating challenging intracellular infections. This brief review intends to highlight and pinpoint in the scientific literature prior studies and references concerning the effects of CPPs that are active against intracellular infectious pathogens, and it explores the antibacterial, antiviral, and antiparasitic properties of several cell-penetrating antimicrobial peptides. To date, CPAPs show potential for druggability in the translational medical application of CPPs either individually or alongside chemotherapeutics. Furthermore, CPAPs might serve as an intriguing option for drug development and the management of intracellular infections.

Keywords: Intracellular Infection; Cell-Penetrating Antimicrobial Peptide; Antimicrobial Peptide; Cell-Penetrating Peptide; Intracellular Pathogen

1. Introduction

Cell-penetrating peptides (CPPs) are brief, typically cationic peptides that can effectively enter eukaryotic cells without causing damage the plasma membrane at internalization concentrations, rendering them promising options for drug delivery applications (Bahnsen, Franzyk et al. 2015). CPPs are recognized for their significant capability to deliver proteins and peptides into cells and through epithelial barriers, whether in vivo and in vitro settings. CPPs possess the distinctive capability to deliver diverse payloads into cells with minimal toxicity, offering significant potential as a robust tool for medical uses. They can carry molecules that typically have restricted intracellular availability because of their hydrophilic nature, overall negative charge, and significant molecular weight. Furthermore, they can facilitate the delivery of nanoparticles, drugs, therapeutic proteins and diagnostic agents (Habault and Poyet 2019). Importantly, CPPs exhibit various physicochemical properties in common with an additional category of bioactive peptides, known as antimicrobial peptides (AMPs). CPPs exhibiting antimicrobial properties, as well as AMPs possessing cell-penetrating abilities, are typically brief amino acid sequences that are amphipathic and possess a net positive charge owing to a large fraction of lysine and arginine residues. The origins of CPPs exhibiting antimicrobial properties are varied: they

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can be chimeric, synthetic, or natural. The range of action of CPPs possessing anti-infective characteristics encompasses efficacy against intracellular viruses, parasites, fungi and Gram-positive and Gram-negative bacteria as examined and elaborated in this discussion. In light of this initial idea, Cell Penetrating Peptides with anti-infective properties emerge as an intriguing option for pharmaceutical drug development aimed at treating intracellular infections (Cruz, Santos et al. 2022). Antibiotics have revolutionized healthcare worldwide since the discovery of the first antibiotic almost a century back. Yet, nearly as swiftly as each antibiotic was available for general use, resistance has overshadowed the therapeutic effectiveness of numerous antimicrobial medications. At present, there are ten approved peptide-based antibacterial, and one lipopeptide, three lipoglycopeptides, comprising two glycopeptides, with slightly more than 40 others in the clinical development stage (Browne et al., 2020). Obstacles to the clinical application of AMP have motivated a move towards peptide mimetics and designed peptides that provide enhanced structural flexibility. These designed peptides could tackle with the stability and toxicity issues of AMPs, enhancing their antimicrobial effectiveness and selectivity (Li and Brimble 2019). The emergence of resistance not only hinders the treatment of bacterial infections but also poses a challenge for antibiotics to achieve their full therapeutic efficacy due to mammalian membrane penetration. For example, choices for addressing intracellular Staphylococcus infections are restricted because many hydrophilic antibiotics, such as glycopeptides and aminoglycosides, have low permeability through mammalian membranes. Consequently, even if an antibiotic works against extracellular bacteria, it could be clear intracellular pathogens that could multiply and cause reinfection. Strategies to address both AMR (antimicrobial resistance) and infections that evade therapy, which emphasize altering current antibiotics, are encouraging. Cell-penetrating peptides (CPPs) may act as a complementary resource to antibiotics in combating difficult bacterial infections. While certain CPP classes might enhance the effectiveness of antibiotics, others could facilitate their transport to specific intracellular sites (Zeiders and Chmielewski 2021).

1.1. Mechanisms of Internalization of Cell-Penetrating Peptides (CPPs)

More than 30 years have elapsed since the discovery of CPPs, and their mechanism of internalization is still not understood. Despite being documented in numerous cell types and alongside various cargoes; the exact mechanism of entry is still unclear. Evaluating the internalization behavior of CPPs is essential for assessing overall efficacy and safety. Additionally, understanding the uptake mechanism may be crucial for synthesis of CPP delivery systems that exhibit minimal toxicity and cell-specificity. The challenge in tackling this problem arises from the intrinsic properties of the peptides, such as their size and charge distribution. These traits allow them to engage with different cell surface molecules, greatly affecting the choice of an entry route (Wang, Wang et al. 2014). The factors mentioned above, only a small sample of numerous ones, steer the internalization pathways of CPPs toward two primary routes: membrane translocation and endocytosis. In general, the kind of uptake that will be chosen largely relies on the physicochemical characteristics of the cargo and the peptide, along with the concentration used, in addition to the structural features of the plasma membrane (Ruseska and Zimmer 2020).

1.2. Endocytosis

Endocytosis is an intricate process consisting of multiple mechanisms and is typically classified into two main types: pinocytosis and phagocytosis. Phagocytosis entails the ingestion of macroparticles and is limited to specific cells (monocytes, macrophages, and neutrophils). Pinocytosis, in contrast, entails the absorption of solutes and fluids and takes place in every cells. A minimum of four different methods have been identified for pinocytosis: CME (clathrin-mediated endocytosis), CvME (macropinocytosis, caveolae-mediated endocytosis), and endocytosis that is independent of caveolae and clathrin (Conner and Schmid 2003) Each of the endocytic processes outlined relies on specific mechanisms and components. To a certain degree, the selection of pathway can be influenced by the types of cells and their differentiation status. Nonetheless, in the case of the Nanocarrier internalization like CPPs, their surface reactivity and physicochemical characteristics also hold significance. It is now broadly recognized that CPPs at low levels, when attached to cargo, are taken up by cells through an energy-dependent process.

In 2003, Richard et al (Richard, Melikov et al. 2003) proposed endocytosis as a mechanism for transporting CPPs through the cellular membranes, citing potential inaccuracies in the findings related to direct translocation attributed to the experimental techniques employed. Many earlier studies, along with more recent research, indicate that macropinocytosis serves as the primary route for CPPs to enter cells (Yesylevskyy, Marrink et al. 2009).

1.3. Direct Translocation

The direct movement of CPPs across the cell membrane as an energy-free process and a substitute to endocytosis was proposed following the observation of CPPs internalization at low temperatures (Trabulo, Cardoso et al. 2010). Direct translocation is viewed as energy - independent process that encompasses a single-phase mechanism and entails the creation of pores, inverted micelles, and the “carpet” model. This procedure can be examined under certain

experimental circumstances – such as low temperature, energy exhaustion, and the application of endocytic inhibitors, for example. In general, for direct translocation to take place, positively charged CPPs need to interact with negatively charged element of the cell membrane, like the phospholipid bilayer, leading to the entry of the CPP. Moreover, direct translocation necessitates a temporary or a permanent disruption of the membrane to enable internalization. It is widely acknowledged that direct translocation takes place at elevated CPP concentrations and is most likely for primary amphipathic CPPs like transportan analogues and MPG (Ruseska and Zimmer 2020).

1.4. Vesicle Budding and Collapse (VBC) Mechanism

The article presents a new mechanism known as vesicle budding and collapse (VBC). This mechanism indicates that CPPs can trigger the development of nucleation sites on the plasma membrane, resulting in vesicle budding. Subsequently, the vesicles disintegrate, enabling the CPPs to move into the cell. The study offers proof for this mechanism by monitoring the actions of certain CPPs (nonaarginine, CPP17 and CPP12) in Jurkat cells through time-lapse confocal microscopy.The VBC mechanism resolves several contradictory findings in the literature concerning CPP internalization (Sahni, Ritchey et al. 2024).

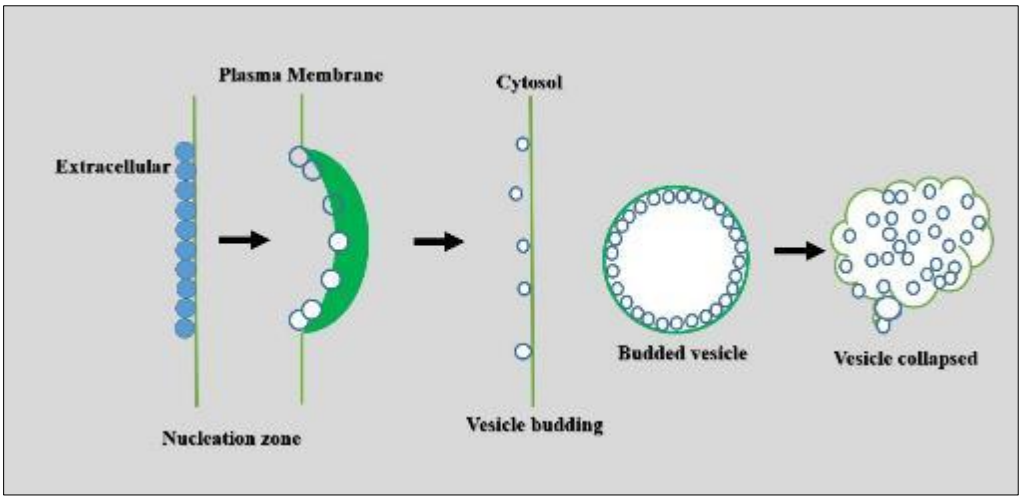

Figure 1 Vesicle Budding and Collapse (VBC)

2. Cell penetrating Peptide stability

The stability of CPPs is an essential aspect for their use in both therapeutics and diagnostics. For CPPs to operate effectively, it is essential that they can carry their cargo to toward the proper location inside the tissue or within the cell without being degraded prematurely by intracellular or extracellular proteases, which would lead to an early release of the cargo. Nonetheless, whether stability is the primary limiting factor relies on the particular the cargo, CPP, its connections to the delivery system, the site of administration, and the specific target location. The impact of enhanced stability has been notably covered in various studies, indicating the deterioration of fluorophore-tagged MAP, Tat, penetratin, and several CPPs derived from human calcitonin (Tréhin, Nielsen et al. 2004) correlated with reduced cellular uptake, suggesting that extracellular metabolism influences peptide uptake. The recent study showed that the pattern of degradation was not influenced by cell type, while the degradation rate matched the enzyme activity levels in the three evaluated cell culture models. It is widely acknowledged that enhanced stability is crucial for the effective CPP-mediated transport of cargo, although a direct link between the translocation efficacy and the degradation of extracellular CPP is not always evident. Intracellular degradation poses a challenge for CPP-mediated cargo delivery, since degradation and sequestration in endocytic vesicles can significantly restrict the delivery of conjugated cargos to the nucleus and cytoplasm. For instance, recent findings indicate that the amount of intact intracellular CPP correlated with the intracellular concentration of free cargo. To prevent lysosomal breakdown of the CPP (and possibly the cargo) by the numerous enzymes found in lysosomes, it is crucial to attain endosomal escape. Lately, Patel et al. examined the possible significance of a cytosolic proteasome-associated degradation pathway concerning the degradation of intracellular CPP (Patel, Zaro et al. 2007). Within this framework, the intracellular handling and breakdown rates of CPPcargo conjugates continue to be a significant area for exploration. Both naturally occurring and synthetic CPP candidates have been altered to enhance stability through modifications such as changing the amino acids from L to D, modifying the peptide sequence, the inclusion of non-α-amino acids, or introducing peptide branching, (Rennert, Wespe et al. 2006) along with the terminal modification . It should be taken into consideration that conjugation to the diagnostic

cargo or drugs may alter the intrinsic stability of peptides that penetrate cells, transporting drugs through membrane barriers. Nonetheless, the enhanced stability of D-peptides compared to L-peptides may not entirely explain the better cellular absorption of the CPP. The CPP–cargo complex must be suitably processed to liberate covalently attached or linked cargo following internalisation to achieve the intended effect. Therefore, the manner of conjugation to the cargo is evidently crucial for attaining the most effective delivery. Certain research indicates that amide or maleimide conjugation exhibits greater stability compared to disulfide binding (Youngblood, Hatlevig et al. 2007).

In certain situations, conditional stability is favored for the best cargo release characteristics. One instance is the use of cargoes and disulfide-bonded CPPs that undergo reduction in the cytoplasm, leading to the liberation of the cargo. When enhancing the transduction effectiveness and stability of CPPs through chemical modification, it is essential to specifically consider the safety issues (e.g., physiological clearance, cellular toxicity, and immunogenicity concerning both short and long-term impacts) (Foged and Nielsen 2008).

2.1. Antimicrobial Activity of Cell-Penetrating Peptides

Antimicrobial peptides (AMPs), dynamic components of the host's inherent immune response to various pathogens, have garnered significant interest as possible substitutes for traditional antibiotics. The majority of AMPs display wide-ranging antimicrobial properties by causing depolarization and permeabilization of the bacterial cytoplasmic membrane. A novel method to improve the antibiotic efficacy of AMPs is through the attachment of a cationic protein transduction domain. Notably, CPP-conjugated AMPs produced merely a 2- to 4-fold rise in microbicidal effectiveness contrary to Gram-positive bacteria, yet demonstrated a 4- to 16-fold enhancement in antimicrobial effectiveness against Gram-negative bacteria.(Lee, Lim et al. 2019). Understanding the mechanism of action is essential to create effective AMPs as new potential drugs. AMPs exert their effects through interactions with cell membrane of microbes, and this interface is significantly influenced by the triglyceride makeup of plasma membranes. (Wu, Patočka et al. 2018). Coulomb force between electropositive and electronegative microbial surfaces facilitates membrane interactions. The Lipoteichoic acids found in the cell wall of Gram-positive bacteria and the lipoglycans present in the Gram-negative bacteria's outer membrane provide a negative electrical charge to bacterial exteriors, enhancing the interrelationship with AMP(Boparai and Sharma 2020). AMPs are categorized according to their mechanism of action into "membrane acting peptides," which break microbial membranes leading to their breakdown, and "non-membrane acting peptides," which can cross membranes without causing damage but disrupt normal cellular functions. (Boparai and Sharma 2020). Three models have been suggested to clarify how AMPs permeabilize bacterial membranes: the barrel stave model, the toroidal-pore model, and the carpet model. (Raheem and Straus 2019).

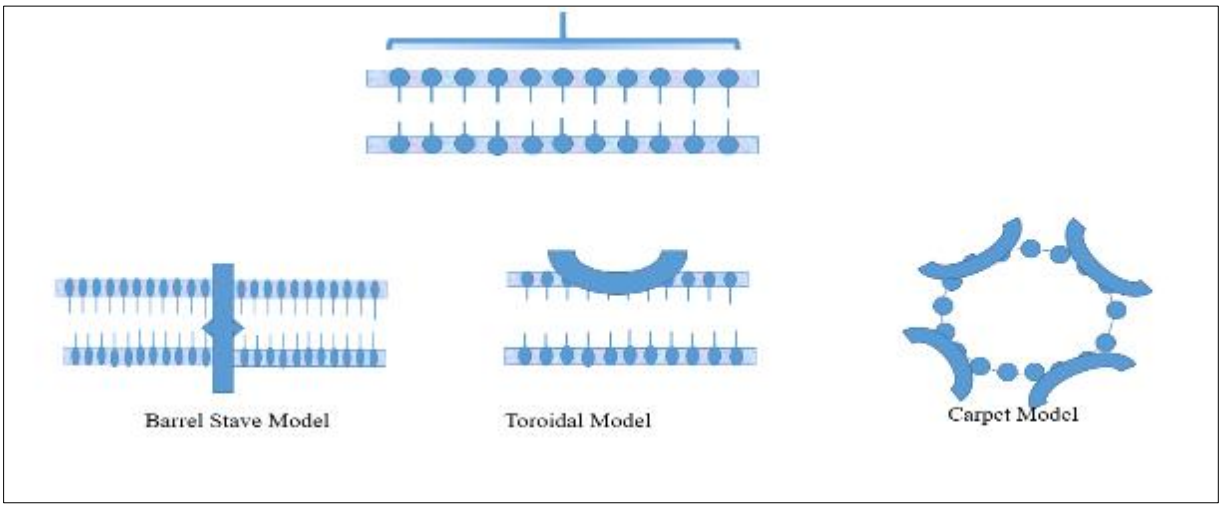

Figure 2 Models to clarify permeabilization of AMPS

AMP insertion can occur perpendicular to the membrane, similar to the barrel-stave model, or like toroidal model at a perpendicular angle while interacting with lipid head groups, causing a refraction in the membrane(Brogden 2005). AMPs can also align parallel to the membrane, entirely surrounding it while concurrently develop micelles with the initially disrupted membranes, in a carpet-like model (Gazit, Miller et al. 1996).

Basically, microorganisms have developed different approaches to outflow the antimicrobial defenses of phagocyte, and once they are consumed by phagocyte, they avoid detection by the host's immune system and the effects of antimicrobial

therapies (Leseigneur, Lê-Bury et al. 2020). The primary challenge for a curative agent to access intracellular bacteria involves traversing the host cell membrane at safe levels. The subsequent challenge arises from the difficulty of reaching microorganisms found in the cytosol of host cells or enclosed within membrane-bound vesicles while maintaining effective antimicrobial activity(Cruz, Santos et al. 2022). Chlamydia trachomatis can endure and reproduce in both inside and outside of the cellular settings. The artificial Pep-1 includes a non-polar motif abundant in tryptophan and a polar domain high in lysine, allowing effective targeting of plasma membranes. Peptide-1 showed an amount-dependent effect on the growth of intracellular C. trachomatis, leading to complete suppression of inclusion creation at a level of 8 mg/L. The sensitivity during the progressive cycle peaked when treatment commenced 12 hours' post-infection. This anti-chlamydial effect could influence the bacterium directly or indirectly by affecting the inclusion of C. trachomatis or the vital host cell processes required for its growth (Park, Yamanaka et al. 2009). Three analogous peptides, belonging to the same group and originating from the original cell penetrating peptide P14LRR, exhibit distinct organelle localizations that facilitate in the directing of harmful bacteria within their cell's environments. This distinct characteristic efficiently removes Enterobacteria and Listeria monocytogenes found within phagocytes. An enhanced organelle localization of P14-5C found in invaginations of membrane could enable this cell penetrating peptides to target Salmonella found in endosomes, leading to better intracellular elimination of the pathogen. The P14-5L peptide builds up in the cytosol of macrophages and greatly decreases the quantity of Enterobacteria present in the groundplasm. The chosen cell penetrating peptides can notably decrease Enterobacteria's infections within the human body, while showing low harmfulness to the insects (Nepal, Mohamed et al. 2018).

One of the many AMPs employed against endosymbionts is plectasin. This is sourced from the fungus Pseudoplectania nigrella displays immuonogenic properties observed in web-spinners, arachnid, damselflies, and bivalves (Mygind, Fischer et al. 2005). Plectasin has been tested against staphylococcal MRSA in both intracellular plus extracellular infection forms (Brinch, Sandberg et al. 2009). Wild-type peptide has been utilized to address THP-1 macrophages infected with Staphylococcus species, resulting to decrease of 1-log load within the cells (Brinch et al., 2009). The plectasin activity found inside cells in vitro was stronger than that of glycopeptide antibiotics (Brinch, Sandberg et al. 2009). Additionally, the plectasin variants NZ2114 and MP1102 were assessed in relation to three strains of Staphylococcus species. In comparison to their plectasin, both analogs considerably decreased the burden within the cells. (Wang, Wang et al. 2014). Additional identical defence antimicrobial peptides, including HNP-1 (a-type human defensins) and RC-1 (humanized q-defensin, retrocyclin-1), were assessed for their effectiveness against intracellular Listeria monocytogenes (Arnett, Lehrer et al. 2011). All these peptides can notably cause a decrease in bacterial growth (Arnett, Lehrer et al. 2011).

Among parasitic diseases, malaria is a serious infectious condition spread by the nibble of the Anopheles mosquito, affecting around one million people annually. Significantly in n recent years, researchers have uncovered antimalarial peptides. Crotamine, a peptide made up of 42 amino acids sourced from the venom of the South American rattlesnake Crotalus durissus terrificus, was examined for its potential to inhibit the Plasmodium falciparum parasite, revealing that this constituent restricts the development of Plasmodium falciparum in a concentration dependent manner. Its impact could be connected to the disturbance of homeostasis within the acidic compartments of Plasmodium. Additionally, the peptide diminished the fluorescence of the dye tracker within the organelle of the parasite, indicating a disruption in the parasite's metabolism (El Chamy Maluf, Dal Mas et al. 2016).

3. Applications of Cell-Penetrating Peptides

The hydrophilic properties of proteins and peptides inhibit their ability to cross cell membranes. Therefore, to effectively transport protein and peptide therapies through endothelial and epithelial barrier or across the plasma membrane, a strategy to enhance permeability should be utilized. CPPs (Cell-penetrating peptides) represent an important resource and have demonstrated potential in delivering proteins and peptides into cells, as well as through blood-brain barrier (BBB) and different epithelia. The transport of proteins and peptides aided by Cell Penetrating Peptides can take place through covalent attachment of the Cell Penetrating Peptides to the cargo protein and peptide, or via physical complexation achieved just by mixing the CPP with its cargo. Each technique has its advantages and disadvantages, and the optimal selection likely depends on the physicochemical characteristics of the Cell Penetrating Peptides and its cargo, along with the administration route, the particular barrier, and the intended cell (Kristensen, Birch et al. 2016). Covalently linking a CPP to a cargo protein and peptide guarantees a close association of the CPP with its cargo, and this can be accomplished chemically via methods such as disulfide bonds (Herce, Deng et al. 2013, Virès, Granier et al. 1997), amine links (Liang and Yang 2005), particularly the connectors (Liang and Yang 2005) that enable the release of the cargo after cellular uptake. Rather than using chemical synthesis, one might utilize an expression host like Saccharomyces or E. coli to create the CPP-cargo conjugate, considering that the viability of chemical production significantly depends on the overall amino acid count in the CPP-fused drug and its folding intricacy (Kristensen, de Groot et al. 2015). Physical complexation through hydrophobic and electrostatic interactions among a

CPP and a cargo protein and peptide can be easily achieved by simple bulk mixing. This method provides adaptability in the utilized CPP-to-cargo molar mixing ratio, while the covalent conjugation technique relies on the presence of intra-sequential or terminal binding sites, crucial for achieving the desired carrier-to-cargo ratio. The formation of non-covalent interactions among the CPP and its cargo relies on the physicochemical characteristics of both the cargo molecules and CPP, alongside the formulation principles utilized (Kristensen, Franzyk et al. 2015). CPPs can also be used to address both local and systemic inflammation resulting from a physical or chemical injury or caused by pathogen invasion. Research has shown that immune cells can absorb the SOC3 (suppressor of cytokine signaling), which reduces inflammation in rats when linked to a CPP derived from FGF4 (fibroblast growth factor 4). Additionally, this CPP-linked suppressor of cytokine signaling was transferred to the liver, where it shielded hepatocytes from apoptosis triggered by virulence factors (Jo, Liu et al. 2005). Cell Penetrating Peptides have been used to boost the production of pluripotent stem cells as safer alternative to traditional methods that require introducing viral genetic material, which carries significant risks for mutagenesis as wee as overall genetic damage, making them unsuitable for human application. Rather, a sequence that does not include arginine was attached to the C-terminal of proteins associated with cellular reprogramming, and the subsequent fusion proteins were effectively taken up by fibroblasts from human infants, which then converted into pluripotent stem cells (Kim, Kim et al. 2009). Inability to efficiently pass through endothelia or epithelia is a major obstacle to the delivery of non-injectable macromolecules or their movement across the BBB. Nonetheless, important applications of CPPs encompass their function as transport vehicles for conveying proteins and peptides through the digestive system (Kamei, Morishita et al. 2008), respiratory epithelia (Patel, Wang et al. 2009), or adenoidal (Khafagy el, Morishita et al. 2009), together with the BBB (Cao, Pei et al. 2002). In 2005, initial research showing CPP-enhanced transepithelial transport of a cargo protein revealed that the covalent attachment of Tat to insulin markedly increased insulin permeability through Caco-2 cell monolayers (Liang and Yang 2005). Since that time, various CPPs have been utilized as vehicles for transepithelial delivery of therapeutic proteins and peptides, with polyarginines serving as transport agents for insulin, gastrin and glucagon-like peptide-1 (GLP-1), (Kamei, Morishita et al. 2009), as well as a vehicle for insulin, GLP-1 and interferon-β (INF-β) (Kristensen, Franzyk et al. 2015). Another use of CPPs is as vehicles for transporting therapeutic proteins and peptides to address neurological disorders, as the delivery of macromolecules to brain targets is significantly limited by the Blood Brain Barrier. In this context, CPPs have demonstrated promise in surmounting this endothelial barrier, such as by linking Tat to Bcl-xl, which inhibits apoptosis in brain neurons. Following the administration of IP to mice, the Tat-Bcl-xl fusion protein was found in different areas of the brain and provided protection from ischemia (Cao, Pei et al. 2002).

Table 1 AMPS in clinical trials.(Hazam, Goyal et al. 2019)

Name	Description	Medical uses
Histatin	Proteins abundant in histidine primarily present in human saliva.	Potent against Candida albicans infections Supportive agent in microbial treatment
h1F1-11	A segment of human lactoferrin, usually made up of 11 amino acids.	Supportive agent in antifungal treatment
Plectasin	Plectasin is a peptide antibiotic that was initially obtained from the f ungus Plectosphaerella cucumerina,	Supportive agent in microbial treatment
Opebacan	Artificial molecule formulated to replicate the structure and role of peptides	Supportive agent in immune therapy
IDR-1	Innatedefense Regulator-1 is a peptide that originates from a broader group of antimicrobial peptides (amps).	Assisting factor in viral infections
P113	Histatin P113 is a segment of the complete human histatin protein,	Assisting factor in viral infections
MX-594AN	A synthetic variant of indolicidin, an antimicrobial peptide that naturally occurs and is sourced from bovine neutrophil Granules,	To treat cathetar-induced acne
PAC113	A segment of histatin peptide,	Potent against Candida albicans infections

CZEN-002	Synthetic derivative of alpha-MSH (alpha-melanocyte-stimulating hormone).	Potent against Candida albicans infections most vulvovaginal infections
EA-230	Fragment of a peptide obtained from beta-human chorionic gonadotropin (β-hcg).	Treatment of septicemia
IMX942	Derivative of Indolicidin, a naturally existing antimicrobial peptide	To treat fever-related and hospital-acquired infections
XOMA-629	A variant of an antimicrobial peptide	To treat sores
RDP58	A peptide obtained from Human Leukocyte Antigen	To treat inherited retinal disorder
NP213	A variant of an antimicrobial peptide	Assisting factor to treat fungal infections
PMX-30063	As an antimicrobial substance with characteristics akin to defensins	Supportive agent in microbial treatment
CLS001	A variant of an Indolicidin,	Supportive agent in anti-inflammatory therapy
OP145	A variant of an antimicrobial peptide	Treatment of septicemia
Ghrelin	Peptide hormone mainly generated in the stomach	Assisting factor in viral infections
AP-214	A variant of alpha-MSH (alpha-melanocyte-stimulating hormone).	To treat pustules
CD-NP	C-type natriuretic peptide	Assisting factor to treat fungal infections
VIP	Vasoactive Intestinal Peptide is essential in modulating the immune system and regulating inflammation.	Supportive agent in viral infections Treatment of septicemia

4. Advantages of cell penetrating peptides

Antimicrobial peptides have been recognized as effective alternatives to traditional antibiotics because of their wide-ranging activity, compatibility with biological systems, and minimal toxicity. Furthermore, they demonstrate activity against MDR (Multi-Drug Resistant) strains and bacterial biofilms, and can work synergistically with traditional antibiotics (Hazam, Goyal et al. 2019).

4.1. Effective against MDR strains

The usefulness of AMPs as a possible substitute for traditional antibiotics was anticipated because of their strong bactericidal effects against biofilm-forming and MDR bacterial strains (Gopal et al., 2014). Peptide molecules such as SMAP-29 have demonstrated efficacy against mutated strains of P. aeruginosa VREF and MDR (Hazam, Goyal et al. 2019).

4.2. Synergism

It has been noted that AMPs used alongside well-known antibiotic classes like nalidixic acid derivatives β-lactams and penams, etc., demonstrated a considerable enhancement in their antimicrobial effectiveness. It has also been studied that chimeric peptides, in combination with traditional ones, were very effective against drug-resistant A. baumannii (Hazam, Goyal et al. 2019). Likewise, it was demonstrated that when used with antibiotics such as tobramycin, imipenem, gentamicin and chloramphenicol, the branched AMP, B2d 088, effectively kills P. aeruginosa synergistically while exhibiting no cytotoxic effects on mammalian cells (Giacometti, Cirioni et al. 2005).

4.3. Biased selectivity and Biocompatibility

AMPs are formed from amino acids, which are organic compounds that occur naturally. This provides a benefit to AMPs due to their direct clearance as natural antibiotics post-clinical trials.The tendency of natural AMPs to target anionic microbial membranes rather than neutral
mammalian membranes is a significant trait that supports the creation of synthetic AMP compounds (Teixeira, Feio et al. 2012).

4.4. Challenges and limitations

The transport of inorganic or organic materials via CPP marks a significant advancement in the area of cellular drug delivery. Nonetheless, significant constraints must also be highlighted. As noted recently and elaborated on further below, associated data, even after being published in reputable life sciences journals, has proven to be affected by artifacts. Furthermore, instances illustrating the therapeutic benefits of the CPP method remain uncommon, and the toxicity issues related to this method have not been adequately addressed. In the end, the ability of CPP to penetrate cellular barriers and the cellular processing that occurs upon interacting with these barriers have, until now, garnered limited focus. Here we will examine a representative range of challenging issues (Tréhin and Merkle 2004).

4.5. Poor cellular uptake

Recent discussions have emphasized the difficulty of inadequate cellular absorption of cell-penetrating peptides (CPPs), like penetratin and Tat, even though they are expected to effectively cross plasma membranes. Research conducted by Koppelhus (Koppelhus, Awasthi et al. 2002) and colleagues indicated that these CPPs exhibited minimal or no internalization in different cell lines. Possible reasons consist of swift degradation or low absorption rate. When employing CPPs to transport biologically active molecules, elevated concentrations were required to achieve noticeable effects, which led to questions regarding their efficacy as delivery agents. Engagement with cell-surface heparans could obstruct the possibility of attaining the cargo release and intended cellular transduction (Falnes, Wesche et al. 2001).

4.6. Cellular uptake is cell line dependent

The cellular absorption of peptides derived from Tat differs considerably depending on the cell line. Although there is considerable evidence indicating that these peptides can penetrate cell membranes of different types, research shows that their uptake depends on particular cell traits. Koppelhus et al. observed that the HIV-1 LTR promoter's activity, frequently utilized to indicate peptide presence, might distort quantitative evaluations (Koppelhus, Awasthi et al. 2002). The authors also showed that modified penetratin(42 –58) and C-Tat(48 – 60) exhibited notable differences in their uptake patterns concerning the evaluated cell line. As a result, they determined that the absorption of the two CPP would be restricted to specific cell types and reliant on lipid compositions or specific molecules on cell membranes. Another study indicated that the cargo delivery and kinetics of cellular absorption via CPP may depend not only on the peptides involved but also on the membrane lipid composition, which differs across various cell lines (Hällbrink, Florén et al. 2001). Additionally, Mai et al. [116] stated that the cell type analyzed influences CPP-mediated transduction (Mai, Shen et al. 2002).

4.7. No permeation through cellular barriers

In research conducted with mice, the intraperitoneal delivery of a fusion protein containing Tat and b-galactosidase exhibited wide distribution, indicating possible systemic penetration of CPPs (cell-penetrating peptides) through barriers, such as the blood-brain barrier. Nonetheless, opposing results by Violini et al. suggested that well-differentiated epithelial cell lines, like MDCK and Caco-2, were primarily impermeable to Tat(Drin, Cottin et al. 2003) under physiological conditions, alike to a paracellular macromolecular marker. Further studies by Mann and Frankel showed that Tat and hCT-derived peptides and have low permeability through different epithelial cells. These findings encourage a reassessment of CPP's capability for systemic drug delivery, emphasizing the possibilities of localized delivery instead (Mann and Frankel 1991).

4.8. Metabolic breakdown of CPP

The metabolic stability of cell-penetrating peptides (CPPs) is essential since they need to transport their cargo prior to degradation. Up to now, only three research studies have investigated CPP metabolism. Elmquist et al. studied pVEC and discovered it quickly deteriorated in murine fibroblasts and human aorta endothelial cells. Nevertheless, substituting L-amino acids with their D-forms strengthened pVEC. The second study examined transportan, TP10, and penetratin, indicating that transportan exhibited greater stability. Additional research from a different laboratory on penetratin and Tat indicated that Tat demonstrates greater stability across three epithelial models, with Calu-3 showing the most peptidase activity. Endopeptidases degraded hCT, implying a consistent metabolic pathway in the models.

Although metabolism can be harmful, it facilitates cargo release and affects CPP clearance and toxicity. Comprehensive CPP design is crucial for achieving a balance among these factors. (Derossi, Chassaing et al. 1998).

5. Future direction

The swift and extensive emergence of drug-resistant pathogens, along with a lower rate of approval for new antibiotic compounds, has led to a rise in mortality rates globally. According to reports, around 25,000 deaths each year in Europe have been noted as a result of infections caused by antibiotic-resistant bacteria's. A comparable number of casualties has also been stated from the U.S, and the death rate in third-world and developing countries is even worse. In total, an annual death toll of 700,000 individuals has been noted, which is expected to reach 10 million by the year 2050 (Cassandra 2017). This scenario requires innovative concepts realized through the creation of advanced molecular structures that can collaboratively address the adaptive mutations caused by resistant bacteria, so peptides may be the simplest and immediate solution to this fundamental issue as we commence the design process. This review emphasizes the recent advancements of AMPs as potential therapeutics along with their related challenges. Nevertheless, AMPs are favored over traditional antimicrobials because of their simple structure, short length, ability to target membranes and stability against proteases. In this aspect, they provide numerous opportunities to enhance their characteristics using methods such as cyclization, heterochiral designs, peptidomimetics, terminus alterations, conjugations, and more. This suggests that AMPs may be created as innovative therapeutic options by addressing their fundamental limitations (Hazam, Goyal et al. 2019).

6. Conclusion

Intracellular bacterial infections present a greater challenge than extracellular infections, because the selected antibacterial agent must penetrate the plasma membrane of host without damaging the cell in order to eradicate the intracellular pathogenic microbes. To effectively treat intracellular infections, high doses of typical antibiotics are generally required due to their limited ability to penetrate cells. Due to these elevated doses, the viability of host cells is frequently compromised. In this summary, we present recent discoveries regarding the use of CPPs and AMPs as alternative therapies for addressing intracellular microbial infections. As outlined, these types of antimicrobials have demonstrated the capability to reach the host cell's intracellular microbes to either perform direct antibacterial actions or transport cargo molecules with antimicrobial properties. Additionally, several studies have shown that these peptides are safe and do not possess any toxic effects on host cells, making them attractive candidates for drugs aimed at treating intracellular infections.

Compliance with ethical standards

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Disclosure of conflict of interest

No potential conflict of interest was reported by the authors.

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